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Published in:
Clinical Microbiology and Infection

DOI:
10.1016/j.cmi.2015.08.022

Publication date:
2016

The population structure of *Staphylococcus aureus* in China and Europe assessed by multiple-locus variable number tandem repeat analysis; clues to geographical origins of emergence and dissemination

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Abstract

To compare the genetic population structure of *Staphylococcus aureus* from China and Europe, 1294 human isolates were characterized by multiple-locus variable number tandem repeat analysis (MLVA). In total, MLVA identified 17 MLVA complexes (MCs), comprising 260 MLVA types (MTs) among the Chinese isolates and 372 MTs among the European isolates. The five most frequent MCs among the Chinese isolates belonged to MC398, MC5 subclade a, MC8, MC437 and MC7 and made up 55% of the sample. For the European isolates, the five most frequent MCs consisted of MC5 subclade a, MC45, MC8, MC30 and MC22, which accounted for 64% of the sample. Phylogeographic analysis of the major MCs shared between China and Europe points to a European origin of MC8 but cannot provide a consistent signal for MC5 subclade a, probably indicating a different origin. Diversity and frequency distributions of other lineages were also compared. Altogether, this study provides the first snapshot of two extant populations of *S. aureus* from Europe and China, and important clues on the emergence and dissemination of different lineages of *S. aureus*.

Keywords: Epidemiology, methicillin-resistant, molecular methods, multiple-locus variable number tandem repeat analysis, *Staphylococcus aureus*

Original Submission: 27 February 2015; **Revised Submission:** 25 July 2015; **Accepted:** 25 August 2015

Editor: G. Lina

Article published online: 3 September 2015

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Introduction

Staphylococcus aureus is a well-known pathogen because of its high virulence potential and its capability of colonizing both humans and domesticated animals. It is the most frequent cause of purulent skin and soft-tissue infections in humans, but it also has the potential to cause life-threatening bloodstream infections, endocarditis, toxic shock syndrome and necrotizing pneumonia. Compared with other bacterial species, the evolution of *S. aureus* is rather clonal [1]. Most isolates of *S. aureus* can be grouped into a limited number of clonal lineages or

http://dx.doi.org/10.1016/j.cmi.2015.08.022

complexes, each of which is defined by highly related core genomes and a unique combination of core variable genes. Whole-genome sequences of *S. aureus* strains reveal that core genes, which exist in all isolates, are highly conserved (97%) and diversify at the level of clonal complexes mainly by the accumulation of single nucleotide substitutions [2–4]. Therefore, it is possible to unequivocally discern most lineages by different molecular typing approaches.

As much attention focused on the emergence and spread of methicillin-resistant *S. aureus* (MRSA), which has become a frequent source of difficult-to-treat hospital-acquired (HA) and community-acquired (CA) infections, most of the available strain collections are biased towards MRSA and very few meaningful conclusions can be inferred about natural populations of methicillin-susceptible *S. aureus* (MSSA) from which MRSA lineages happen to emerge. The present study therefore aimed to describe the genetic structure of large *S. aureus* population samples collected at multiple sites in China and Europe using comprehensive sampling frames that reflect the natural diversity of *S. aureus*.

Materials and Methods

Bacterial isolates

In order to gather a representative sample of the *S. aureus* population, 1294 *S. aureus* isolates were included in the study. Of these isolates, 647 were collected in China during the years 2005–2011, and comprised 253 clinical and 394 healthy carriage isolates (85.5% MSSA, 14.5% MRSA) (Table 1). Isolates from patients were collected from two hospitals in eastern China, and healthy carriage isolates originated from 1530 and 918 nose swabs that were sampled at health centres in Beijing and Harbin, respectively. For comparison, an equivalent number of European *S. aureus* isolates (n = 647, 64.9% MSSA, 35.1% MRSA) were randomly selected from the European survey database of isolates from the Staphylococcal Reference Laboratory collected in 2006 and 2007 in 25 countries (Table 2) [5].

TABLE 1. Overview of 647 Chinese *Staphylococcus aureus* isolates collected from various locations and sources between 2005 and 2011

Isolate source	Province	No. of isolates	No. of MSSA	No. of MRSA
Patients	Anhui	190	133	57
	Zhejiang	63	32	31
Healthy carriers	Heilongjiang	188	183	5
	Beijing	206	205	1
Total		647	553	94

Abbreviations: MRSA, methicillin-resistant *Staphylococcus aureus*; MSSA, methicillin-susceptible *S. aureus*.

Nasal swabs from healthy carriers were enriched in tryptic soy broth (OXOID, Basingstoke, UK) with 7% NaCl at 37°C for 24 h, and then plated onto mannitol salt agar (OXOID). Plates were incubated at 37°C for 24 h. Presumptive *S. aureus* colonies were identified by coagulase production using the Slidex Staph Plus kit (Murex Biotech Ltd., Dartford, UK), and confirmed as *S. aureus* by *nuc* PCR [6]. The presence of the *mecA* gene was used to define MRSA [6]. The clinical isolates were obtained from diagnostic hospital laboratories and were also subjected to *nuc* and *mecA* PCR for confirmation purposes.

Genetic characterization of isolates

MLVA typing was performed as described by Schouls *et al.* [7]. All of the isolates were characterized using *spa* typing and representative isolates of MLVA types (MT) were subjected to multilocus sequence typing for the assignment of clonal complexes (CC) [8,9].

The Panton–Valentine leukocidin genes (*lukS*-PV and *lukF*-PV) were detected by PCR as previously described [10].

Data analysis

Minimum spanning trees were used to illustrate the genetic relationships (BIONUMERICS, beta version 3.5; Applied Maths, Kortrijk, Belgium). For assignment of MLVA complexes, the entire in-house MLVA-type database (available at www.mlva.net) was interrogated. The MTs were grouped if they differed by no more than a single variable number tandem repeat whereby the MT with the largest number of single locus variants (SLVs) was regarded as the founder. Groups of four or

TABLE 2. Overview of 647 European *Staphylococcus aureus* isolates collected from different countries in 2006–2007

Isolate source	Country	No. of isolates	No. of MSSA (%)	No. of MRSA (%)
Patients	Austria	49	35 (8.3)	14 (6.2)
	Belgium	25	3 (0.7)	22 (9.7)
	Bulgaria	14	6 (1.4)	8 (3.5)
	Croatia	17	12 (2.9)	5 (2.2)
	Cyprus	2	2 (0.5)	0 (0.0)
	Czech Republic	45	28 (6.7)	17 (7.5)
	Denmark	25	25 (5.9)	0 (0.0)
	Finland	11	7 (1.7)	4 (1.8)
	France	71	35 (8.3)	36 (15.9)
	Germany	47	26 (6.2)	21 (9.3)
	Greece	3	1 (0.2)	2 (0.9)
	Hungary	9	8 (1.9)	1 (0.4)
	Iceland	1	1 (0.2)	0 (0.0)
	Ireland	57	29 (6.9)	28 (12.3)
	Italy	37	21 (5.0)	16 (7.1)
	Netherlands	54	51 (12.1)	3 (1.3)
	Norway	20	20 (4.8)	0 (0.0)
	Poland	44	36 (8.6)	8 (3.6)
	Portugal	32	18 (4.3)	14 (6.2)
	Slovenia	22	19 (4.5)	3 (1.3)
	Spain	62	37 (8.8)	25 (11.0)
Total		647	420 (100.0)	227 (100.0)

Abbreviations: MRSA, methicillin-resistant *Staphylococcus aureus*; MSSA, methicillin-susceptible *S. aureus*.

more MTs were regarded as MLVA complexes (MCs). Chi-squared statistics (SPSS, SPSS Inc., Chicago, IL, USA) were used to test for differences in proportions. A p-value of ≤ 0.05 was considered statistically significant. MLVA types within clonal complexes that have the largest numbers of variants were defined as founders. Distance to founder in MC8 and MC subclade 5a between Chinese and European isolates was calculated by the number of isolates that differ from a founder by one locus (i.e. SLVs), two (double locus variants, DLV), three (triple locus variants, TLV), etc.

RIDOM EPICOMPARE software version 1.0 (Ridom GmbH, Würzburg, Germany) was used to calculate the discriminatory power at the MLVA level in each MC. The discriminatory power was estimated by Simpson's index of diversity, expressing the probability that two unrelated and different isolates sampled from the test population would be grouped into different subtypes by a specific typing method. Non-overlapping CI were regarded as representing statistically significant differences in discriminatory power.

Results

The MLVA identified 260 MTs and 372 MTs among the Chinese and European isolates, respectively. Both Chinese and European isolates were clustered into 17 distinct MCs (Fig. 1). There was a systematic difference in the distribution of these *S. aureus* complexes between China and Europe (Fig. 1, Table 3).

Eight MLVA complexes had significantly more Chinese isolates. These were MC1, MC5 subclade b, MC7, MC123, MC398, MC437, MC621 and MC1933. In contrast, isolates from European patients were dominant in other MCs, namely MC2, MC5 subclade a, MC22, MC30 and MC45. The five most frequent MCs among the Chinese isolates were MC398, MC5 subclade a, MC8, MC437 and MC7, which made up 55.0% of the Chinese sample. For the European isolates, the five most frequent MCs were MC5 subclade a, MC45, MC8, MC30 and MC22, which accounted for 64.3% of the sample. Hence the five most frequent complexes in China and in Europe had only MC8 and MC5 subclade a in common, which together also contained most of the MRSA (62%) in the entire collection. Sixty-six per cent of all MC8 isolates, and 49% of all MC5 subclade a were MRSA (Table 3). Distances to founder in MC8 and MC5 subclade a were compared between Chinese and European isolates (Table 4). Fig. 2 offers a detailed view of the tree structure of MC8 and MC5. In MC8, the majority of European variants occupy a central position and either belong to the founder MLVA type or are SLVs and DLVs of the founder, whereas Chinese isolates are DLVs and TLVs and do not have founder MLVA types. European isolates also occupy many more branches indicating a higher diversity than Chinese isolates (see Supplementary material, Table S2). The distributions of Chinese and European variants of MC5 subclone a are very similar in terms of branch length, branch distribution and distance to founder. Also there is an equal degree of genetic diversity between isolates from both origins.

The remaining MRSA isolates from China and Europe—not belonging to either MC8 and MC5 subclade a—were widely distributed over 22 MTs and 93 MTs, respectively (Fig. S1A, C), but two MCs contained significantly more MRSA than others. These were MC2 and MC22. MC2 consisted of only MRSA and all of the isolates originated from Europe. Also all of the MRSA isolates within complex MC22 (80%) were from Europe. A list of the top five MCs of MSSA and MRSA isolates and the corresponding multilocus sequence typing and *spa* types is provided in the Supplementary material (Tables S1a (MSSA) and S1b (MRSA)).

A comparison of the genetic diversity shows that Chinese isolates and European isolates do not differ at the level of individual MCs except for MC8 (see Supplementary material, Table S2). The same applies to Chinese clinical and Chinese carriage isolates if only MCs that contain more than ten isolates are considered.

The presence of *pvl* genes was determined for all the Chinese isolates. Thirty-eight isolates (5.9%) were *pvl* positive, which include five MRSA and 33 MSSA isolates. The majority of isolates belonging to MC5 subclade b/CC88 (70%) were *pvl* positive.

Discussion

Our present study represents the first attempt to describe two extant populations of *S. aureus* from opposite ends of the same continental shelf—China and Europe. Using MLVA as a typing method for isolates obtained through dedicated surveys, we found a systematic difference in the distribution of *S. aureus* clonal lineages between Europe and China. This difference was particularly pronounced for four international lineages (MC2, MC22, MC30 and MC45) that are dominant in Europe and also include successful lineages of hospital-acquired (HA-)MRSA (MC2 and MC22). Compared with the European isolates, eight MLVA complexes (MC1, MC7, MC123, MC398, MC437, MC621, MC1933 and MC5 subclade b) were found to be dominant among the Chinese isolates. The comparison with the European isolates suggests that the Chinese sample consists of a combination of pandemic lineages and a considerable number of local clones. MC8/ST239/CC8 and MC5/ST5/CC5 are clear representatives of pandemic MRSA lineages in China, which were also shown in previous studies [11]. Both lineages are also

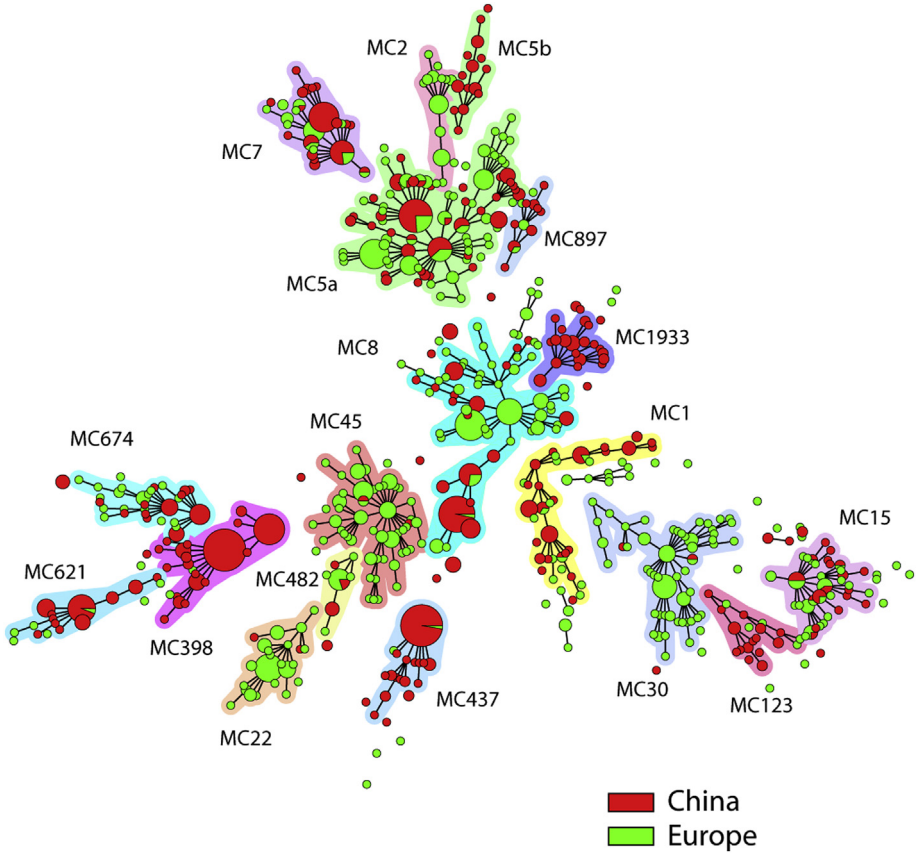

FIG. 1. Minimum spanning tree of *Staphylococcus aureus* isolates from China and Europe based on the relatedness between multiple-locus variable number tandem repeat analysis (MLVA) types. Each circle represents a single MLVA type, and the size of the circle indicates the number of isolates with the same MLVA type. Geographical origin (China or Europe) is indicated by red or green segments respectively. MLVA complexes are shown by coloured halos.

widely prevalent in Europe [12] and contained the majority of MRSA of the present sample.

It should be noted that there are clear limitations to this study, mainly due to unequal sampling. The Chinese *S. aureus* sample was obtained from two source populations, whereby the majority of isolates (n = 394) were from healthy carriers while only 253 were clinical isolates. This is in stark contrast to the European sample, which was entirely made up of clinical isolates. Moreover, the sampling frame used for the European collection deliberately enriched for MRSA [5]. This explains the larger proportion of MRSA in the European sample and could account for a degree of overrepresentation of MCs that typically include MRSA. A recent study carried out among healthy carriers in nine European nations revealed a frequency distribution of the main lineages among MSSA and MRSA that was almost indistinguishable from the frequency distribution of the European sample used in our present study [13]. It can therefore be assumed that the spectrum and diversity of MRSA and MSSA reflect the natural distribution of lineages in Europe despite the enrichment of nosocomial MRSA in the European sample.

We also argue that clinical samples are representative of carriage isolates as it has been shown that most of the patients, who developed *S. aureus* infection after admission to a hospital, were infected by their own colonizing strains [14]. However, carriage isolates recovered from hospital patients may differ from carriage isolates in healthy carriers from the community, as the former have had more exposure to health care and antibiotic treatment and are therefore more likely—over time—to acquire and carry typical hospital-associated strains. Indeed we found that Chinese clinical isolates were more likely to be MRSA and clustered among different MLVA types than isolates recovered from healthy carriers in the community (see Supplementary material, Fig. S1). This differential distribution cannot be investigated for European isolates, which were all from infected patients.

Another incongruence that deserves consideration relates to the geodemographic representativeness of the samples used in

TABLE 3. Distribution of MLVA complexes (MCs) of methicillin-sensitive and methicillin-resistant *Staphylococcus aureus* by China and Europe

		Frequency(%) of MSSA			Frequency(%) of MRSA			Frequency(%) overall		
MLVA complex	Clone complex (CC)	China	Europe	p	China	Europe	p	China	Europe	p
1	1	38 (6.9)	10 (2.4)	0.001	3 (3.2)	0	0.025	41 (6.3)	10 (1.5)	<0.0001
2	5	0	0	–	0	28 (12.3)	<0.0001	0	28 (4.3)	<0.0001
5a	5	58 (10.5)	37 (8.8)	0.445	20 (21.3)	72 (31.7)	0.077	78 (12.1)	109 (16.9)	0.018
5b	88	20 (3.6)	0	<0.0001	0	0	–	20 (3.1)	0	<0.0001
7	7	52 (9.4)	28 (6.7)	0.128	1 (1.1)	0	0.293	53 (8.2)	28 (4.3)	0.006
8	8	22 (4.0)	33 (7.9)	0.011	52 (55.3)	54 (23.8)	<0.0001	74 (11.4)	87 (13.4)	0.312
15	15	24 (4.3)	33 (7.9)	0.027	0	0	–	24 (3.7)	33 (5.1)	0.278
22	22	2 (0.4)	10 (2.4)	0.006	0	39 (17.2)	<0.0001	2 (0.3)	49 (7.6)	<0.0001
30	30	2 (0.4)	78 (18.6)	<0.0001	0	2 (0.9)	1	2 (0.3)	80 (12.4)	<0.0001
45	45	1 (0.2)	73 (17.4)	<0.0001	1 (1.1)	17 (7.5)	0.029	2 (0.3)	90 (13.9)	<0.0001
123	121	21 (3.8)	5 (1.2)	0.015	0	0	–	21 (3.3)	5 (0.8)	0.002
398	398	89 (16.1)	1 (0.2)	<0.0001	3 (3.2)	0	0.025	92 (14.2)	1 (0.1)	<0.0001
437	1	59 (10.7)	2 (0.5)	<0.0001	0	0	–	59 (9.1)	2 (0.3)	<0.0001
482	8	5 (1.0)	9 (2.1)	0.172	2 (2.1)	2 (0.9)	0.583	7 (1.1)	11 (1.7)	0.478
621	59	35 (6.3)	3 (0.7)	<0.0001	6 (6.4)	1 (0.4)	0.003	41 (6.3)	4 (0.6)	<0.0001
674	25	29 (5.2)	19 (4.5)	0.656	0	0	–	29 (4.5)	19 (2.9)	0.185
897	20	12 (2.2)	4 (0.9)	0.203	0	0	–	12 (1.8)	4 (0.6)	0.075
1933	ST6[a]	34 (6.2)	0	<0.0001	0	0	–	34 (5.3)	0	<0.0001
None		50 (9.0)	75 (17.9)	–	6 (6.4)	12 (5.3)	–	56 (8.7)	87 (13.4)	–
Total		553 (100.0)	420 (100.0)	–	94 (100.0)	227 (100.0)	–	647 (100.0)	647 (100.0)	–

Abbreviations: MLVA, multiple-locus variable number tandem repeat analysis; MRSA, methicillin-resistant *Staphylococcus aureus*; MSSA, methicillin-susceptible *S. aureus*.
[a]ST6 is a double locus variant (DLV) of ST5.

this study. The Chinese isolates were collected in far fewer locations than the European isolates (Tables 1 and 2). However, healthy carriers sampled in Beijing were from 31 of the 34 provincial level divisions of China and included, apart from ethnic Han Chinese, also individuals from 16 of the 55 other ethnic minorities in China. This shows that the population snapshot from Beijing was hardly local, but rather representative of a much larger geographical region. Furthermore, a recent study on the diversity of *S. aureus* bacteraemia isolates from 16 hospitals in 12 cities in China showed a similar distribution of lineages, whereby ST239 and ST5 were the main MRSA genotypes, while CC7, CC188, CC5 and CC398 were the dominant MSSA genotypes [15]. We therefore believe that the results of our present study were not biased to the extent that it would invalidate the overall conclusions, which can highlight the differences in the distribution of major clonal lineages in China and Europe.

TABLE 4. Frequencies of n-locus-variants in MLVA complex (MC) subclade 5a and MC8 isolates from Europe and China

	Number of isolates (number of MLVA-types)			
	MC5a from Europe	MC5a from China	MC8 from Europe	MC8 from China
Founder	5 (1)	8 (1)	15 (1)	–
One locus different from founder	29 (12)	34 (10)	37 (9)	5 (1)
Two loci different from founder	48 (22)	18 (11)	17 (13)	10 (5)
Three loci different from founder	21 (12)	17 (10)	11 (8)	46 (6)
Four loci different from founder	6 (4)	1 (6)	7 (4)	4 (2)
Five loci different from founder	–	–		9 (2)
Total no. isolates	109	78	87	74

Abbreviations: MLVA, multiple-locus variable number tandem repeat analysis.

The analysis of the distances to founder in MC8 showed the majority of European variants are SLVs and DLVs of the founder, whereas more Chinese variants are DLVs and TLVs. MLVA diversity in MC8 was also markedly higher in Europe than in China. The higher diversity is an indication of longer evolutionary time spans during which lineages had time to diversify locally while limited diversity indicated recent introduction. Hence our results point to a European origin of MC8.

The MC5 subclade a corresponds to CC5, which is a common and widespread clonal complex. The analysis of the distances to founder and MLVA diversity suggest that this lineage may have emerged neither in Europe nor in China. Instead, it appears to have diversified independently in both locations along different trajectories caused by geographic segregation. This hypothesis is supported by investigating the evolutionary history of ST5-MRSA, which showed that SCC*mec* acquisition occurs frequently in local *S. aureus* populations, whereas the long-distance geographical spread of MRSA is relatively rare [16]. MC5 subclade b is a Chinese MSSA clade and most of the respective isolates are Panton–Valentine leukocidin positive. Multilocus sequence typing analysis of this clade showed that all of these isolates belong to two novel sequence types, namely ST2148 and ST2141, which are both SLVs of ST88 (CC88). ST88 was reported to prevail over a large geographical area in Africa and was also frequently isolated in Australia but is sporadically reported in Europe [17,18].

MSSA MC398/ST398/CC398 represented the most frequently identified human colonizer in China, encompassing 16% of all MSSA isolates of the Chinese sample. Consistent with this observation, ST398/CC398 MSSA strains have also been reported previously as major causative agents for HA-MSSA and CA-MSSA infections in China [19,20]. Our own previous observations also showed that isolates of this lineage are frequently found among slaughter pigs in the Heilongjiang province [21]. In Europe, the ST398-MRSA lineage is mainly associated with livestock, and it is occasionally transmitted to humans during occupational exposure. It has furthermore been suggested that livestock-associated-ST398 MRSA is mostly transmitted among veterinarians and their families rather than being transmitted in hospitals [22,23]. In contrast, the ST398 MSSA clone found in China seems to be readily transmitted between humans, both in hospitals and in the community [19,20]. In the present study, we found two major MTs belonging to MC398, namely MT565/MC398/ST398/CC398/t571 and MT569/MC398/ST398/CC398/t034 (Fig. S1B). Interestingly, MT569 isolates were also identified in pigs (data not shown), whereas MT565 isolates were so far exclusively isolated from humans. This finding suggests that these two main subclones of MC398 in China may have become specifically adapted to different hosts. In this context it is noteworthy that in recent years an increasing number of ST398/CC398/t571 MSSA infections have been reported in geographically diverse regions around the globe [24]. It still remains to be seen if these isolates are monophyletic and represent a public health challenge in Europe and China.

MC621 was another complex that probably originated from and disseminated in China. One of the SLVs of MT621/MC621/ST2147/t437 is MT1035/MC621/ST59/t437. E-burst analysis showed that ST2147 does not belong to any known clonal complex. Different lineages occasionally share the same *spa* gene indicative of homoplasy within the *spa* region. MRSA isolates belonging to ST59/CC59 were previously identified as the most common type of CA-MRSA in Chinese children with skin and soft-tissue infections. Importantly, ST59/CC59/t437 was also reported as the predominant CA-MRSA type in other Asian countries [25], and it was recently identified across

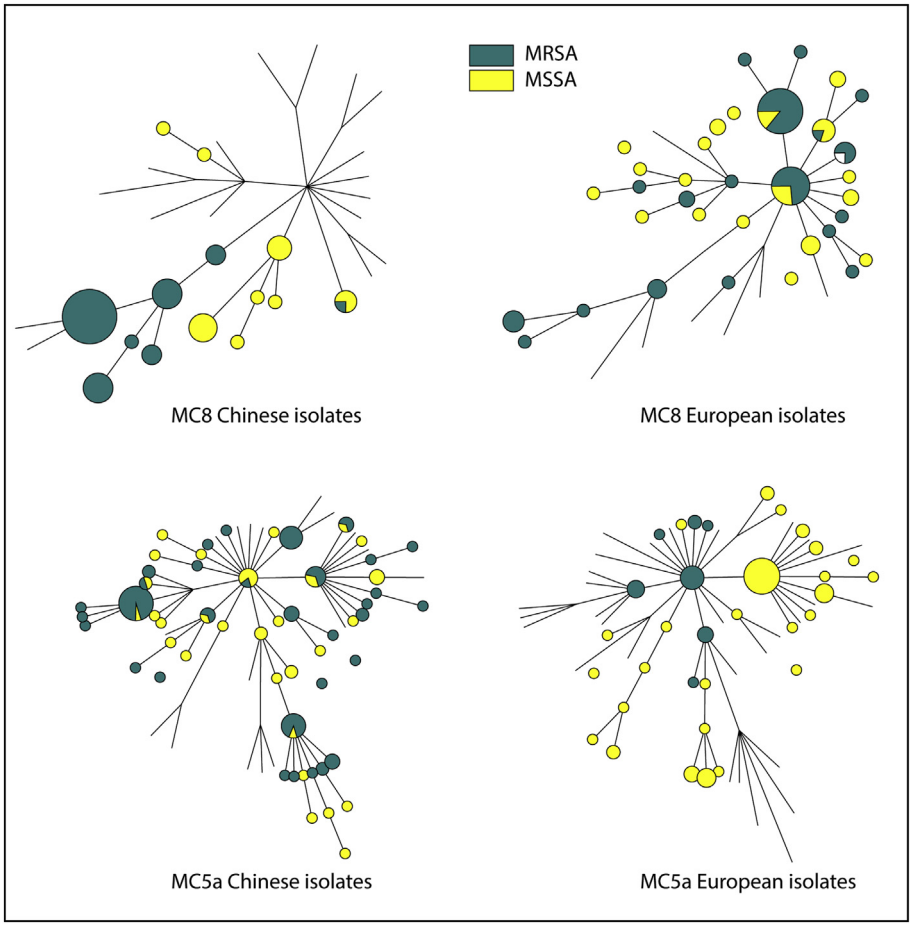

FIG. 2. Minimum spanning tree displaying the methicillin-resistant *Staphylococcus aureus* (MRSA)/methicillin-susceptible *S. aureus* (MSSA) distribution among Chinese and European isolates belonging to MC8 and MC5a. Each circle represents a single multiple-locus variable number tandem repeat analysis (MLVA) type, and the size of the circle indicates the number of isolates with the same MLVA type. Resistance characteristics: MRSA versus MSSA are indicated in dark green and yellow colour code. Note that branches without nodes indicate MLVA types that are absent from either the Chinese or European isolates.

Europe [26]. Whether this predominant CA-MRSA clone has evolved from MC621/ST2147/t437 still needs to be elucidated. It remains also to be seen whether the *spa* type t437 confers an advantage in terms of transmissibility or tenacity, which would explain the abundance of this *spa* type among community-acquired MRSA in China and its rapid expansion in Europe [26].

Some lineages, such as MC2, MC22, MC30 and MC45, which represent successful clones in Europe hardly exist in China. MC2 is related to the southern German HA-MRSA clone (ST228/CC5), which was reported in southern Germany and Italy in the mid-1990s [27,28], and which has spread through the Alpine, Balkan and Appenine regions in recent years [18]. MC22/ST22/CC22 is the most successful European MRSA clone. One reason for its apparent absence from China could be that MC22/ST22/CC22 has not yet had sufficient time to diffuse in China as it did in Europe, where it emerged as EMRSA-15 in the late 1980s in the UK [2]. Furthermore, MC30/CC30 and MC45/CC45 were only found in the European sample. This finding is in accordance with previous studies, where the two complexes were confined to Europe and the USA [29].

In conclusion, the present study provides novel hypotheses about the geographical origins of emergence and events leading to the dissemination of clonal lineages of *S. aureus* with particular public health importance in China and Europe. As such, the results will serve as leads for future studies to better understand the evolution of the *S. aureus* globally.

Transparency Declaration

The authors declare that they have no conflicts of interest.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (Grant No. 81301463), by the State Key Laboratory for Infectious Disease Prevention and Control Fund of China (2012SKLID202), and by an educational grant from the Graduate School of Medical Sciences of the University of Groningen, the Netherlands. Isolates from Europe were collected through a joint effort of the European Staphylococcal Reference Laboratory Working Group. The contribution of Karina Krziwanek, Sabine Stumvoll, Walter Koller, Olivier Denis, Marc Struelens, Dimitr Nashev, Ana Budimir, Smilja Kalenic, Despo Pieridou-Bagatzouni, Vladislav Jakubu, Helena Zemlickova, Henrik Westh, Anders Rhod Larsen, Robert Skov, Frederic Laurent, Jerome Ettienne, Birgit Strommenger, Wolfgang Witte, Sofia Vourli, Alkis Vatopoulos, Anni Vainio, Jaana Vuopio-Varkila, Miklos Fuzi, Erika Ungvári, Stephan Murchan, Angela Rossney, Edvins Miklasevics, Arta Balode, Gunnsteinn Haraldsson, Karl G. Kristinsson, Monica Monaco, Annalisa Pantosti, Michael Borg, Marga van Santen-Verheuvel, Xander Huijsdens, Lillian Marstein, Trond Jacobsen, Gunnar Skov Simonsen, Marta Aires-de-Sousa, Herminia de Lencastre, Agnieszka Luczak-Kadlubowska, Waleria Hryniewicz, Monica Straut, Irina Codita, Maria Perez-Vazquez, Jesus Oteo Iglesias, Vesna Cvitkovic Spik, Manica Mueller-Premru, Sara Haeggman, Barbro Olsson-Liljequist, Matthew Ellington, Angela Kearns, is gratefully acknowledged.

Appendix A. Supplementary data

Supplementary data related to this article can be found online at http://dx.doi.org/10.1016/j.cmi.2015.08.022.

References

[1] Feil EJ, Cooper JE, Grundmann H, Robinson DA, Enright MC, Berendt T, et al. How clonal is *Staphylococcus aureus*? J Bacteriol 2003;185:3307–16.
[2] Holden MT, Hsu LY, Kurt K, et al. A genomic portrait of the emergence, evolution, and global spread of a methicillin-resistant *Staphylococcus aureus* pandemic. Genome Res 2013;23:653–64.
[3] Lindsay JA, Holden MT. Understanding the rise of the superbug: investigation of the evolution and genomic variation of *Staphylococcus aureus*. Funct Integr Genomics 2006;6:186–201.
[4] Lindsay JA. Genomic variation and evolution of *Staphylococcus aureus*. Int J Med Microbiol 2010;300:98–103.
[5] Grundmann H, Aanensen DM, van den Wijngaard CC, et al. Geographic distribution of *Staphylococcus aureus* causing invasive infections in Europe: a molecular-epidemiological analysis. PLoS Med 2010;7:e1000215.
[6] Brakstad OG, Aasbakk K, Maeland JA. Detection of *Staphylococcus aureus* by polymerase chain reaction amplification of the *nuc* gene. J Clin Microbiol 1992;30:1654–60.
[7] Schouls LM, Spalburg EC, van LM, et al. Multiple-locus variable number tandem repeat analysis of *Staphylococcus aureus*: comparison with pulsed-field gel electrophoresis and *spa*-typing. PLoS One 2009;4:e5082.
[8] Harmsen D, Claus H, Witte W, et al. Typing of methicillin-resistant *Staphylococcus aureus* in a university hospital setting by using novel software for *spa* repeat determination and database management. J Clin Microbiol 2003;41:5442–8.
[9] Enright MC, Day NP, Davies CE, et al. Multilocus sequence typing for characterization of methicillin-resistant and methicillin-susceptible clones of *Staphylococcus aureus*. J Clin Microbiol 2000;38:1008–15.
[10] Lina G, Piemont Y, Godail-Gamot F, et al. Involvement of Panton-Valentine leukocidin-producing *Staphylococcus aureus* in primary skin infections and pneumonia. Clin Infect Dis 1999;29:1128–32.
[11] Liu Y, Wang H, Du N, et al. Molecular evidence for spread of two major methicillin-resistant *Staphylococcus aureus* clones with a unique geographic distribution in Chinese hospitals. Antimicrob Agents Chemother 2009;53:512–8.

[12] Harris SR, Feil EJ, Holden MT, et al. Evolution of MRSA during hospital transmission and intercontinental spread. Science 2010;327:469–74.
[13] den Heijer CD, van Bijnen EM, Paget WJ, et al. Prevalence and resistance of commensal *Staphylococcus aureus*, including meticillin-resistant *S. aureus*, in nine European countries: a cross-sectional study. Lancet Infect Dis 2013;13:409–15.
[14] von EC, Becker K, Machka K, et al. Nasal carriage as a source of *Staphylococcus aureus* bacteremia. Study Group. N Engl J Med 2001;344:11–6.
[15] He W, Chen H, Zhao C, et al. Population structure and characterisation of *Staphylococcus aureus* from bacteraemia at multiple hospitals in China: association between antimicrobial resistance, toxin genes and genotypes. Int J Antimicrob Agents 2013;42:211–9.
[16] Nubel U, Roumagnac P, Feldkamp M, et al. Frequent emergence and limited geographic dispersal of methicillin-resistant *Staphylococcus aureus*. Proc Natl Acad Sci U S A 2008;105:14130–5.
[17] Moremi N, Mshana SE, Kamugisha E, et al. Predominance of methicillin resistant *Staphylococcus aureus* -ST88 and new ST1797 causing wound infection and abscesses. J Infect Dev Ctries 2012;6:620–5.
[18] Monecke S, Coombs G, Shore AC, et al. A field guide to pandemic, epidemic and sporadic clones of methicillin-resistant *Staphylococcus aureus*. PLoS One 2011;6:e17936.
[19] Chen H, Liu Y, Jiang X, et al. Rapid change of methicillin-resistant *Staphylococcus aureus* clones in a Chinese tertiary care hospital over a 15-year period. Antimicrob Agents Chemother 2010;54:1842–7.
[20] Zhao C, Liu Y, Zhao M, et al. Characterization of community acquired *Staphylococcus aureus* associated with skin and soft tissue infection in Beijing: high prevalence of PVL+ ST398. PLoS One 2012;7:e38577.
[21] Yan X, Yu X, Tao X, et al. *Staphylococcus aureus* ST398 from slaughter pigs in northeast China. Int J Med Microbiol 2014;304:379–83.
[22] Bootsma MC, Wassenberg MW, Trapman P, et al. The nosocomial transmission rate of animal-associated ST398 meticillin-resistant *Staphylococcus aureus*. J R Soc Interface 2011;8:578–84.
[23] Bosch T, Verkade E, van LM, et al. Transmission and persistence of livestock-associated MRSA among veterinarians and their household members. Appl Environ Microbiol 2015;81:124–9.
[24] Rasigade JP, Laurent F, Lina G, et al. Global distribution and evolution of Panton-Valentine leukocidin-positive methicillin-susceptible *Staphylococcus aureus*, 1981–2007. J Infect Dis 2010;201:1589–97.
[25] Song JH, Hsueh PR, Chung DR, et al. Spread of methicillin-resistant *Staphylococcus aureus* between the community and the hospitals in Asian countries: an ANSORP study. J Antimicrob Chemother 2011;66:1061–9.
[26] Glasner C, Pluister G, Westh H, et al. *Staphylococcus aureus spa* type t437: identification of the most dominant community-associated clone from Asia across Europe. Clin Microbiol Infect 2015;21:163–8.
[27] Wisplinghoff H, Bischoff T, Tallent SM, et al. Nosocomial bloodstream infections in US hospitals: analysis of 24,179 cases from a prospective nationwide surveillance study. Clin Infect Dis 2004;39:309–17.
[28] Mato R, Campanile F, Stefani S, et al. Clonal types and multidrug resistance patterns of methicillin-resistant *Staphylococcus aureus* (MRSA) recovered in Italy during the 1990s. Microb Drug Resist 2004;10:106–13.
[29] Melles DC, Tenover FC, Kuehnert MJ, et al. Overlapping population structures of nasal isolates of *Staphylococcus aureus* from healthy Dutch and American individuals. J Clin Microbiol 2008;46:235–41.